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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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BIOASSAY OF

LITHOCHOLIC ACID

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
U.S. National Cancer Institute
" National Institutes of Health
Bethesda, Maryland 20014

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REPORT ON THE BIOASSAY OF LITHOCHOLIC ACID
FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of lithocholic acid conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of lithocholic acid was conducted by Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. F. M. Garner (4) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly. Chemical analysis was performed by Midwest Research Institute (6) and the analytical results were reviewed by Dr. N. Zimmerman (7).

Histopathologic examinations were performed by Dr. B. C. Zook (4) at Litton Bionetics, Inc., the pathology narratives were written by Dr. B. C. Zook (4), and the diagnoses included in this report represent the interpretation of this pathologist. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (8).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (9); the statistical analysis was performed by Mr. R. M. Helfand (7) and Dr. J. P. Dirkse, III (10) using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (11).

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SUMMARY

A bioassay for the possible carcinogenicity of lithocholic acid was conducted using Fischer 344 rats and B6C3F1 mice. Lithocholic acid was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species, except for 49 low dose female rats. Twenty animals of each sex and species were placed on test as controls. The high and low dosages of lithocholic acid administered were, respectively, 500 and 250 mg/kg for rats and 250 and 125 mg/kg for mice. The compound was administered to rats and mice for 103 weeks. The period of compound administration was followed by an observation period of 1 week for rats and 2 weeks for mice.

There were no significant positive associations between the dosages of lithocholic acid administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Slight dose-related mean body weight depression was observed in male rats and female mice and high incidences of chronic kidney inflammation were observed in female rats, indicating that the dosages of lithocholic acid administered to these animals in this bioassay may have approximated the maximum tolerated dosages. Since no mean body weight depression, relative to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of lithocholic acid to male mice, it is possible that these animals may have been able to tolerate a higher dosage. However, in the subchronic study there were deaths among all the dosed male mouse groups, even those receiving lithocholic acid at a level only twofold greater than the high dose utilized in the chronic study.

None of the statistical tests for any site in rats or in mice of either sex indicated a significant positive association between compound administration and tumor incidence.

Under the conditions of this bioassay, lithocholic acid was not carcinogenic when administered by gavage to Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

Lithocholic acid (Figure 1) (NCI No. C03861), a naturally occurring bile acid, was selected for bioassay by the National Cancer Institute because it has been reported to promote the development of hepatoma and hyperplastic nodules induced by DL-ethionine in rat liver (Hiasa et al., 1971), and because of the strong correlation between concentrations of neutral sterols and bile acid derivatives in human feces and the incidence of human colon cancer (Hill et al., 1971).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is (3α,5β)-3-hydroxycholan-24-oic acid.* It is also called 3α-hydroxy-5β-cholan-24-oic acid; 3α-hydroxy-5β-cholanic acid; 3α-hydroxychloanic acid; 3-monohydroxycholanic acid; and 17β-(1-methyl-3-carboxypropyl)ethiocholan-3α-ol.

Lithocholic acid and a number of other bile acids occur in human feces in concentrations which vary with the predominant dietary composition of the individual or individuals from whom samples are taken. In one study, the following average daily levels of excreted lithocholic acid were measured: among 17 Americans on a mixed Western diet high in fat and animal protein, 81.1 $\pm$ 12.9 mg/day; among 11 American Seventh-Day Adventists on a mixed Western diet without meat, 15.0 $\pm$ 4.1 mg/day; among 12 American strict vegetarians, 23.4 $\pm$ 4.7 mg/day; among 21 Japanese-Americans on a Japanese diet, 22.8 $\pm$ 3.3

*The CAS registry number is 434-13-9.

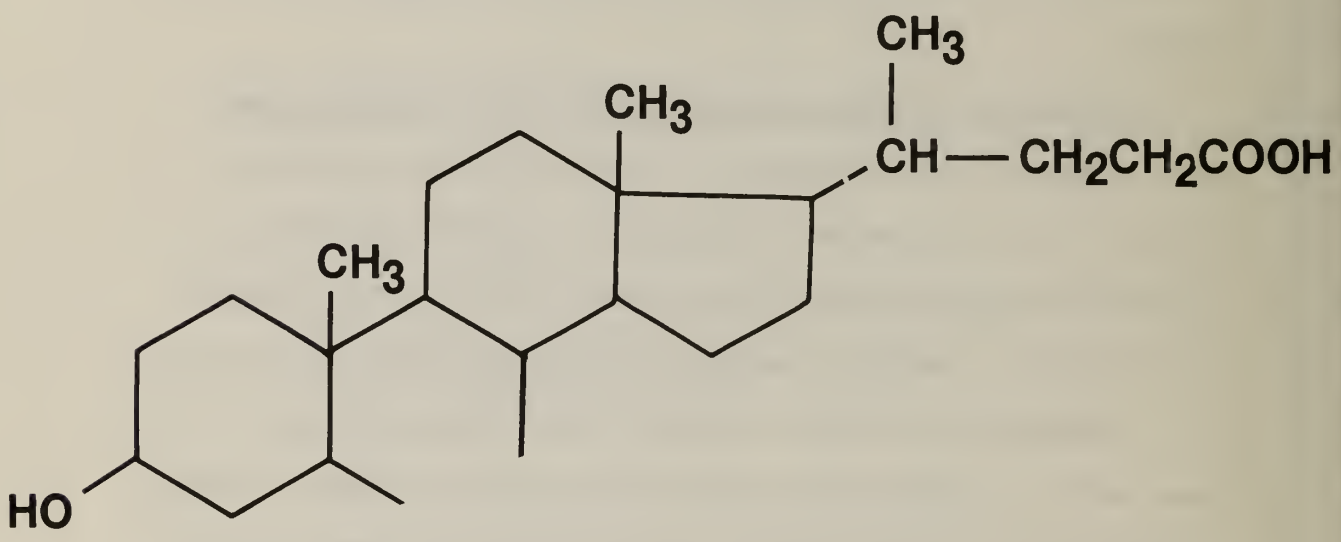


FIGURE 1
CHEMICAL STRUCTURE OF LITHOCHOLIC ACID

mg/day; and among 11 Chinese-Americans on a Chinese diet, 20.0 ± 5.7 mg/day (Reddy and Wynder, 1973). The high levels of lithocholic and other bile acids in fecal samples from Americans show a strong correlation with the significantly increased incidence of colon cancer among this population over that of Japanese and Seventh-Day Adventists. No comparable data are available for Chinese or American vegetarian populations (Reddy and Wynder, 1973).

Further evidence for a connection between lithocholic acid and colon cancer is the increased level of this compound in the feces of colon cancer patients (5.7 ± 1.0 mg/g dry feces) over that of controls (3.4 ± 0.02 mg/g dry feces) (Reddy et al., 1975).

Lithocholic acid does not appear to be produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually) by any U.S. companies (U.S. International Trade Commission, 1977). Lithocholic acid is produced in smaller quantities for biological research, either by purification from bile or by chemical modification of the related bile acids deoxycholic acid and cholic acid (Hawley, 1977).

Lithocholic acid has been shown to exert tumor-promoting activity in two mammalian studies. Hepatocellular carcinoma was found in the liver of 9 of 12 male Wistar rats surviving 20 to 34 weeks after initiation of a diet containing 0.5 percent lithocholic acid and 0.1 percent DL-ethionine, but was found in only 3 of 12 surviving rats receiving 0.1 percent DL-ethionine alone. No carcinoma was found in

9 control rats, 14 receiving 0.5 percent lithocholic acid, 13 receiving 0.05 percent DL-ethionine, or 12 receiving 0.5 percent lithocholic acid plus 0.05 percent DL-ethionine (Hiasa et al., 1971). In a second study with Charles River CD-Fischer rats of both sexes, lithocholic acid increased the frequency of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced colorectal neoplasms after intrarectal administration. Neoplasms were found in 15 of 29 rats receiving one intrarectal dose of 4 mg MNNG followed by intrarectal administration of 1 mg lithocholic acid 5 times weekly for 13 months. Administration of MNNG alone induced neoplasms in 8 of 32 rats. No neoplasms were found in 32 rats receiving only lithocholic acid (Narisawa et al., 1974).

II. MATERIALS AND METHODS

A. Chemicals

Lithocholic acid was purchased from California Biochemical Corporation, Los Angeles, California. Chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. The melting point (184° to 186°C) compared favorably with the literature values reported (Berger et al., 1955 [183° to 184.5°C]; Heusser and Wuthier, 1947 [187° to 188°C]; Fischer, 1911 [184°C]). The results of elemental analysis of the compound were within 1 percent of that expected on a theoretical basis. Thin-layer chromatography was performed utilizing two solvent systems (i.e., ethyl acetate and acetone:benzene). Each plate was visualized with methyl red and each indicated the presence of three spots, one major spot and two impurities. Vapor-phase chromatography yielded two peaks, one approximately 10 percent of the area of the second. The results of infrared and nuclear magnetic resonance analyses were consistent with those expected based upon the structure of the compound and agreed with the literature spectra (Fischmeister, 1960; Small et al., 1969). Ultraviolet analysis revealed λ_{max} at 209 nm with a molar extinction coefficient of 67. No literature value was found for comparison. Virtually all (i.e., 100 $\pm$ 2 percent) of the material responded to titration of the acid function.

Throughout this report, the term lithocholic acid is used to represent this material.

B. Dosage Preparation

Fresh solutions of lithocholic acid in shelf-grade A&P corn oil (Great Atlantic and Pacific Tea Company, Baltimore City, Maryland) were prepared on each day that intubation was performed. Excess portions of the mixtures were disposed of rather than stored. The concentration of lithocholic acid in corn oil ranged from 5 to 10 percent for rats, and from 2.5 to 5 percent for mice.

Dosed corn oil preparations containing 48 and 100 mg/ml of lithocholic acid were analyzed spectrophotometrically. The mean result immediately after preparation was 94 percent of theoretical (ranging from 90 to 98 percent).

C. Animals

The two animal species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats were supplied by the Frederick Cancer Research Center, Frederick, Maryland, and Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. All mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

Rats and mice were approximately 4 weeks old when received. Upon receipt, animals were examined and any obviously ill or runted animals were killed. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were placed on test at this time. Animals

were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

All animals were housed by species in rooms with a temperature range of 22° to 26°C and a range in relative humidity of 45 to 55 percent. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia) at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

All rats were housed four per cage by sex and all mice were housed five per cage by sex. Throughout the study dosed and control animals of both species were housed in polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) suspended from aluminum racks. Racks were fitted with a continuous piece of stainless steel mesh over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and bedding were provided twice weekly. Ab-sorb-dri® hardwood chip bedding (Wilner Wood Products Company, Norway, Maine) was used in polycarbonate cages for the entire bioassay.

Acidulated water (pH 2.5) was supplied to animals in water bottles. Water bottles were changed and washed twice weekly, and sipper tubes were washed at weekly intervals. All animals were supplied with Wayne Lab-Blox® meal in hanging stainless steel hoppers which

were refilled three times per week and sanitized weekly. Food and water were available ad libitum for both species.

All dosed and control rats were housed in a room with other rats receiving diets containing* EDTA trisodium salt (150-38-9); rats receiving I.P. injections of methiodal sodium (126-31-8); and other rats intubated with pivalolactone (1955-45-9).

All dosed and control mice were housed in a room with mice receiving diets containing N,N'-diethylthiourea (105-55-5); EDTA trisodium salt (150-38-9); 3,3'-dimethoxybenzidine-4,4'-diisocyanate (91-93-0); triphenyltin hydroxide (76-87-9); carbromal (77-65-6); diaminozide (1596-84-5); p-quinone dioxime (105-11-3); and 4-amino-2-nitrophenol (119-34-6); and other mice receiving I.P. injections of methiodal sodium (126-31-8).

E. Gastric Intubation

Intubation was performed for three days per week on a mg/kg body weight basis, utilizing the most recently observed group mean body weight as a guide for determining the dose. All animals were weighed and dosages adjusted once monthly, based on group mean body weight. Thus, although the ratio of dose to weight remained constant, the total dose administered changed with an increase or decrease in group mean body weight. Animals of each sex within a dosed group received the same dosage.

*CAS registry numbers are given in parentheses.

F. Selection of Initial Dose Levels

To establish the dosages of lithocholic acid for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. During the first week of the subchronic study, lithocholic acid was incorporated into the basal laboratory diet and supplied ad libitum to five of the six groups of each species in concentrations of 6800, 10,000, 14,700, 21,600 and 31,500 ppm. The remaining group of each species served as a control group, receiving only the basal laboratory diet.

Due to the instability of lithocholic acid in feed, the chemical was administered by gavage beginning in week 2 of the subchronic test. The dosages utilized were 464, 681, 1000, 1470, and 2150 mg/kg and were, respectively, administered to the groups initially receiving 6800, 10,000, 14,700, 21,600, and 31,500 ppm. Intubation was performed 3 times per week for 7 weeks, followed by a 1-week observation period. The control group received corn oil by gavage from weeks 2 through 8. Individual body weights and food consumption data were recorded twice weekly throughout the study. Upon termination of the study all survivors were sacrificed and necropsied.

The following table indicates the mean body weight gain, relative to controls, and survival observed in each of the dosed rat groups at the end of the subchronic test.

RAT SUBCHRONIC STUDY RESULTS

Dosage (mg/kg)	Mean Body Weight Gain (%)*		Survival**	
	Males	Females	Males	Females
2150	-20	+3	5/5	5/5
1470	-15	+6	5/5	5/5
1000	- 8	+8	5/5	5/5
681	- 6	+4	5/5	5/5
464	-11	+4	5/5	5/5
0	--	--	5/5	5/5

No other clinical signs were recorded for any rat group. The high dose selected for administration to dosed rats in the chronic bioassay was 500 mg/kg.

The following table indicates the mean body weight gain, relative to controls, and survival observed in each of the dosed mouse groups at the end of the subchronic test.

MOUSE SUBCHRONIC STUDY RESULTS

Dosage (mg/kg)	Mean Body Weight Gain (%)*		Survival**	
	Males	Females	Males	Females
2150	--	--	0/5	0/5
1470	--	--	0/5	0/5
1000	--	--	0/5	0/5
681	+ 7	- 7	2/5	4/5
464	+16	-13	4/5	5/5
0	--	--	5/5	5/5

No other clinical signs were recorded for any mouse group. The high dose selected for administration to dosed mice in the chronic bioassay was 250 mg/kg.

*+ is indicative of mean body weight gain greater than that of controls
- is indicative of mean body weight gain less than that of controls.

**Number of animals observed/number of animals originally in group.

G. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, dosages administered, and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dosages of lithochlolic acid administered to rats were 500 and 250 mg/kg. Throughout this report those rats receiving the former dosage are referred to as the high dose groups and those receiving the latter dosage are referred to as the low dose groups. Dosed rats were administered lithocholic acid for 103 weeks followed by a 1-week observation period.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dosages of lithocholic acid administered were 250 and 125 mg/kg. Throughout this report those mice receiving the former dosage are referred to as the high dose groups and those receiving the latter dosage are referred to as the low dose groups. Dosed mice were administered lithocholic acid for 103 weeks followed by a 2-week observation period.

Vehicle control animals were intubated with 5 ml/kg corn oil three times per week for 103 weeks and received no intubations for the remaining 2 weeks of the bioassay.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
LITHOCHOLIC ACID GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	LITHOCHOLIC ACID DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
VEHICLE CONTROL	20	0	0	104
LOW DOSE	50	250 0	103	1
HIGH DOSE	50	500 0	103	1
FEMALE				
VEHICLE CONTROL	20	0	0	104
LOW DOSE	49	250 0	103	1
HIGH DOSE	50	500 0	103	1

[a]Dosages, given in mg/kg body weight, were administered by gavage 3 days per week.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
LITHOCHOLIC ACID GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	LITHOCHOLIC ACID DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
VEHICLE CONTROL	20	0	0	105
LOW DOSE	50	125	103	
		0		2
HIGH DOSE	50	250	103	
		0		2
FEMALE				
VEHICLE CONTROL	20	0	0	105
LOW DOSE	50	125	103	
		0		2
HIGH DOSE	50	250	103	
		0		2

[a]Dosages, given in mg/kg body weight, were administered by gavage 3 days per week.

H. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. Body weights for rats were recorded at monthly intervals throughout the bioassay. Body weights for mice were recorded once a week for the first 6 weeks, every 2 weeks for the next 4 weeks, and at monthly intervals thereafter. All animals were inspected twice daily. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group.

All moribund animals or animals that developed large, palpable masses that jeopardized their health were killed. A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was killed at the end of the bioassay. The animals were euthanized with carbon dioxide, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in a 10 percent neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney,

urinary bladder, pituitary, adrenal, thyroid, parathyroid, seminal vesicle, testis, prostate, brain, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were recorded in each group at the time that the test was initiated.

I. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control

animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at

the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality

between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

Slight dose-related mean body weight depression was apparent in male rats throughout a major portion of the bioassay. Dosed female rats, however, did not evidence mean body weight depression relative to controls (Figure 2).

No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female rats in the control and lithocholic acid-dosed groups are shown in Figure 3. The Tarone test did not indicate a significant positive association between dosage and mortality for rats of either sex. Similarly, the Cox tests comparing the dosed groups to the control were also not significant.

There were adequate numbers of male rats at risk from late-developing tumors as 80 percent (40/50) of the high dose, 90 percent (45/50) of the low dose, and 80 percent (16/20) of the controls survived on test until the termination of the study.

There was also an adequate number of female rats at risk from late-developing tumors, as 66 percent (33/50) of the high dose, 82 percent (41/50) of the low dose, and 80 percent (16/20) of the controls survived on test for at least 104 weeks.

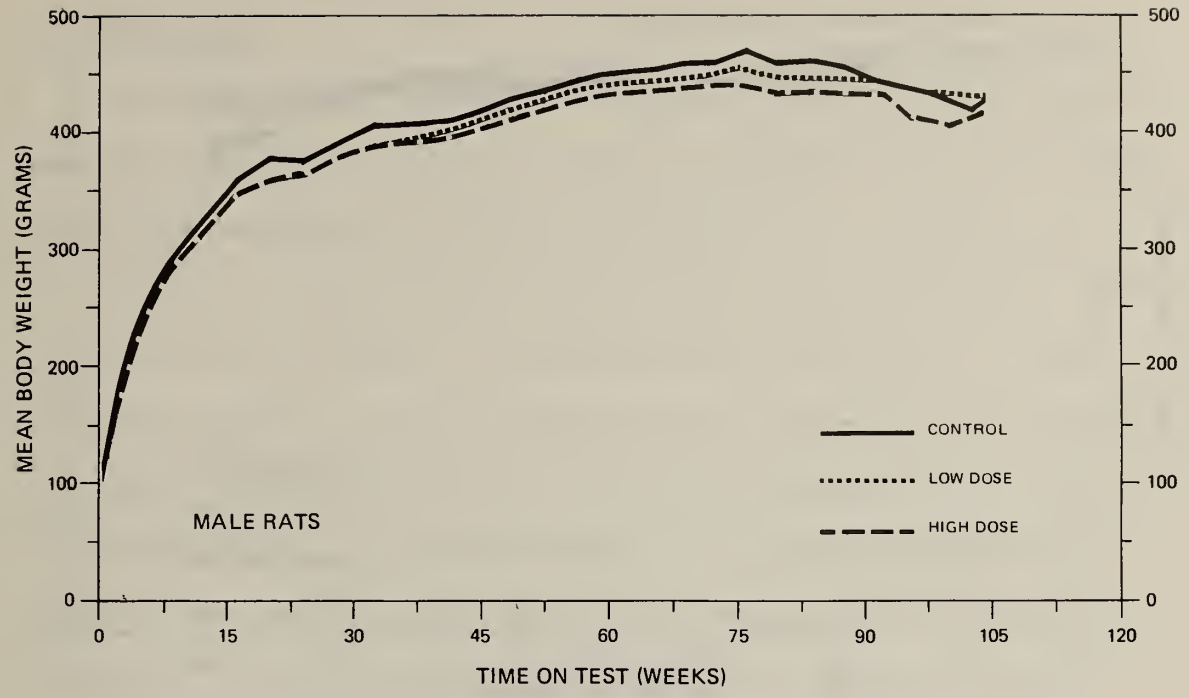


FIGURE 2
GROWTH CURVES FOR LITHOCHOLIC ACID CHRONIC STUDY RATS

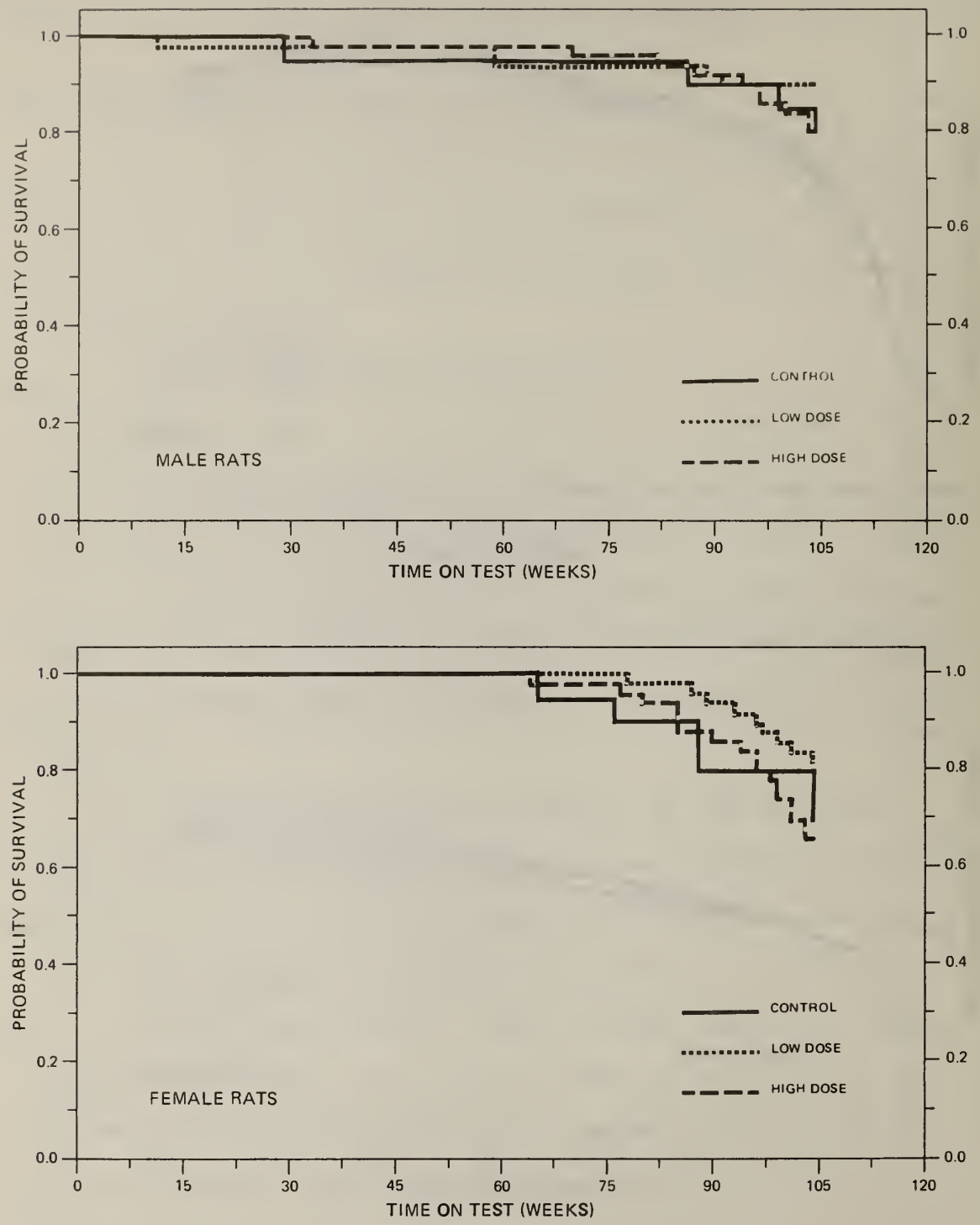


FIGURE 3
SURVIVAL COMPARISONS OF LITHOCHOLIC ACID CHRONIC STUDY RATS

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are summarized in Appendix C (Tables C1 and C2).

There was a spontaneous occurrence of a variety of tumors in the control and dosed groups. A few neoplasms occurred only, or with a greater frequency, in rats in dosed groups as compared with controls. One adenocarcinoma of the colon and one adenocarcinoma of the small intestine were seen in high dose males. The neoplasms which were observed have all been reported to occur spontaneously at similar incidences in this strain of rats. None of the neoplasms were considered compound-related.

A number of inflammatory and degenerative lesions were encountered both in control and dosed rats. The lesions are all well-recognized as spontaneous in older rats of this strain. Chronic inflammation of the kidneys was especially common and observed more often in dosed rats, suggesting a positive relation to the compound, especially in female rats.

Based on the results of this pathologic examination, lithocholic acid was not carcinogenic to male or female Fischer 344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE RATS TREATED WITH LITHOCHOLIC ACID[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	1/20(0.05)	4/50(0.08)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.600	0.400
Lower Limit	---	0.175	0.005
Upper Limit	---	77.169	30.802
Weeks to First Observed Tumor	104	89	104
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	4/50(0.08)	5/50(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.800	1.000
Lower Limit	---	0.128	0.184
Upper Limit	---	8.436	10.007
Weeks to First Observed Tumor	99	91	96
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	0/19(0.00)	1/49(0.02)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.021	0.238
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	103

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	0/19(0.00)	5/47(0.11)	6/46(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.533	0.691
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	104
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	3/20(0.15)	7/50(0.14)	12/50(0.24)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.933	1.600
Lower Limit	---	0.245	0.503
Upper Limit	---	5.215	8.185
Weeks to First Observed Tumor	104	104	103
Thyroid: Follicular-Cell Carcinoma or Follicular-Cell Adenoma[b]	0/09(0.00)	2/39(0.05)	1/27(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.076	0.020
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	104

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Adenoma[b]	0/09(0.00)	4/39(0.10)	1/27(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.242	0.020
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	103
Pancreatic Islets: Islet-Cell Adenoma[b]	2/20(0.10)	3/50(0.06)	2/50(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.600	0.400
Lower Limit	---	0.076	0.032
Upper Limit	---	6.860	5.277
Weeks to First Observed Tumor	99	89	104
Testis: Interstitial-Cell Tumor[b]	19/20(0.95)	45/49(0.92)	47/50(0.94)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.967	0.989
Lower Limit	---	0.900	0.922
Upper Limit	---	1.173	1.168
Weeks to First Observed Tumor	86	59	70

TABLE 3 (CONCLUDED)

[a]Treated groups received doses of 250 or 500 mg/kg by gavage 3 times/week.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH LITHOCHOLIC ACID[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/20(0.15)	11/49(0.22)	13/50(0.26)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.497	1.733
Lower Limit	---	0.460	0.556
Upper Limit	---	7.741	8.773
Weeks to First Observed Tumor	65	87	77
Pituitary: Chromophobe Adenoma[b]	10/20(0.50)	18/46(0.39)	21/48(0.44)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.783	0.875
Lower Limit	---	0.443	0.510
Upper Limit	---	1.585	1.732
Weeks to First Observed Tumor	76	87	64
Adrenal: Pheochromocytoma[b]	0/20(0.00)	3/47(0.06)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.266	0.022
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	93	104

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	1/20(0.05)	6/49(0.12)	6/50(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.449	2.400
Lower Limit	---	0.332	0.325
Upper Limit	---	110.166	108.021
Weeks to First Observed Tumor	104	104	85
Uterus: Endometrial Stromal Polyp[b]	1/20(0.05)	7/48(0.15)	7/50(0.14)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.917	2.800
Lower Limit	---	0.420	0.403
Upper Limit	---	128.374	123.407
Weeks to First Observed Tumor	104	104	77

[a]Treated groups received doses of 250 or 500 mg/kg by gavage 3 times/week.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or lithocholic acid-dosed groups and where such tumors were observed in at least 5 percent of the group.

None of the statistical tests indicated a significant positive association between the administration of lithocholic acid and an increased tumor incidence at any site for rats of either sex. Thus, at the dose levels used in this experiment, there was insufficient evidence to conclude that lithocholic acid was a carcinogen in Fischer 344 rats.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by lithocholic acid that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

No dose-related mean body weight depression was apparent in male mice. There was slight mean body weight depression after week 45, when dosed females were compared to controls (Figure 4).

No other clinical signs were recorded.

B. Survival

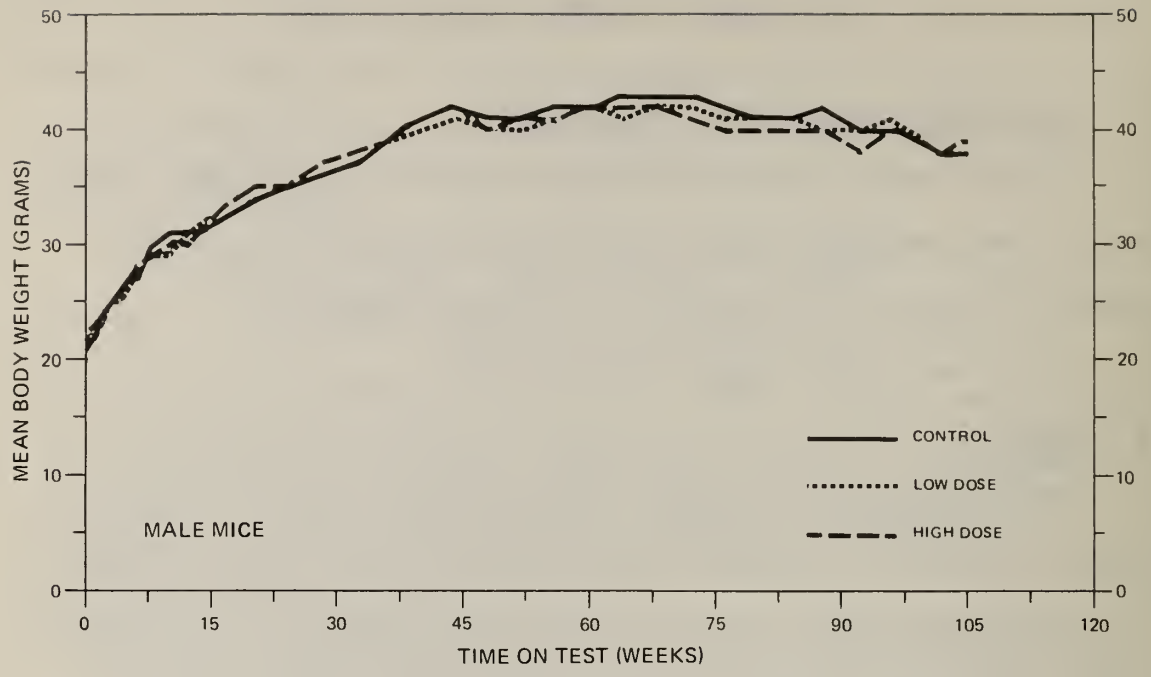
The estimated probabilities of survival for male and female mice in the control and lithocholic acid-dosed groups are shown in Figure 5. Neither the Tarone test nor the Cox tests indicated a significant positive association between dosage and mortality in either male or female mice.

There were adequate numbers of male mice at risk from late-developing tumors, as 64 percent (32/50) of the high dose, 74 percent (37/50) of the low dose and 75 percent (15/20) of the controls survived on test for at least 105 weeks.

An adequate number of female mice were at risk from late-developing tumors as 82 percent (41/50) of the high dose, 68 percent (34/50) of the low dose and 65 percent (13/20) of the controls survived on test until the termination of the study.

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are summarized in Appendix D (Tables D1 and D2).



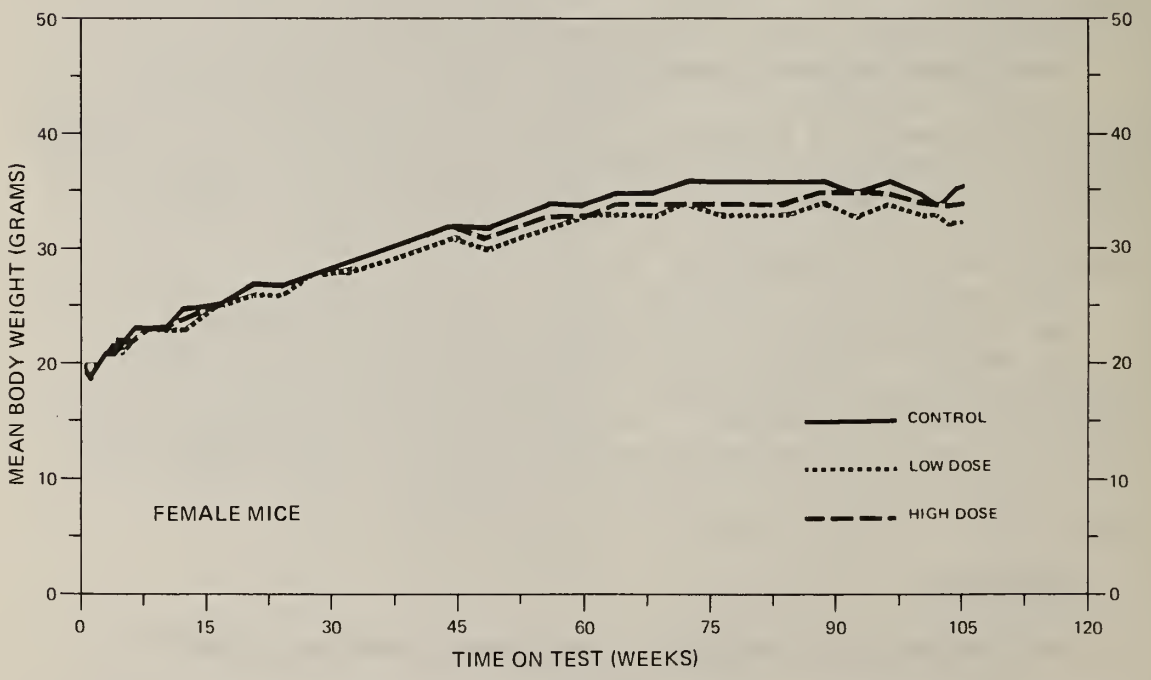
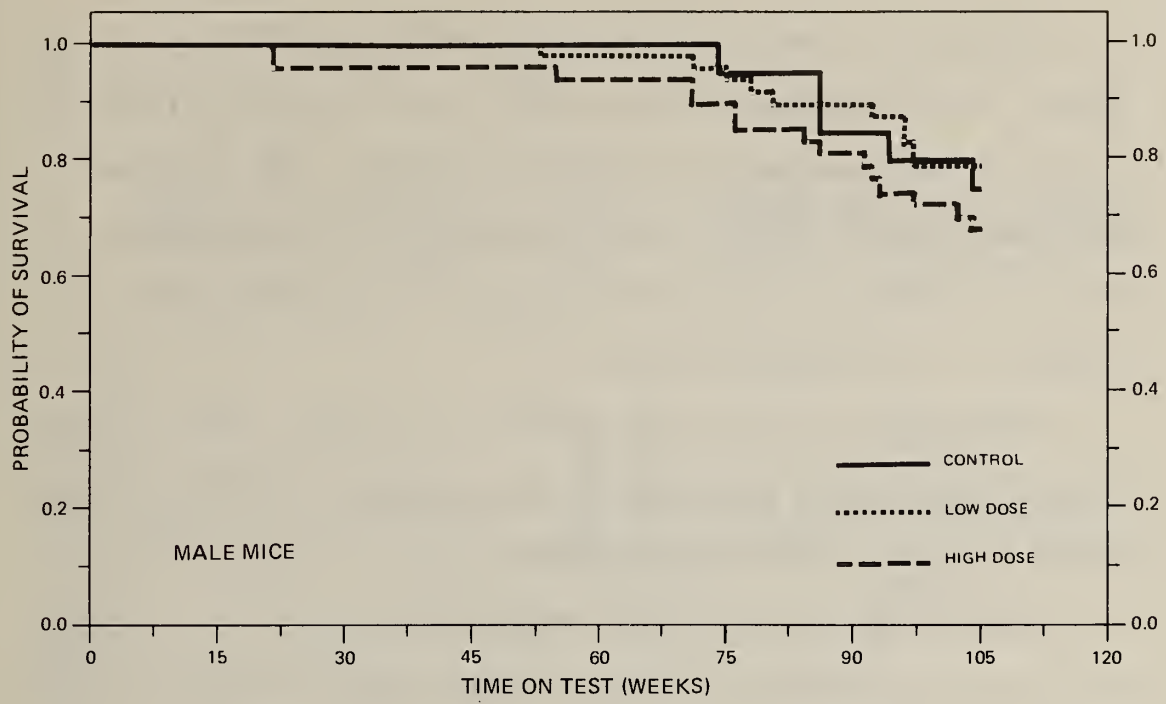


FIGURE 4
GROWTH CURVES FOR LITHOCHOLIC ACID CHRONIC STUDY MICE



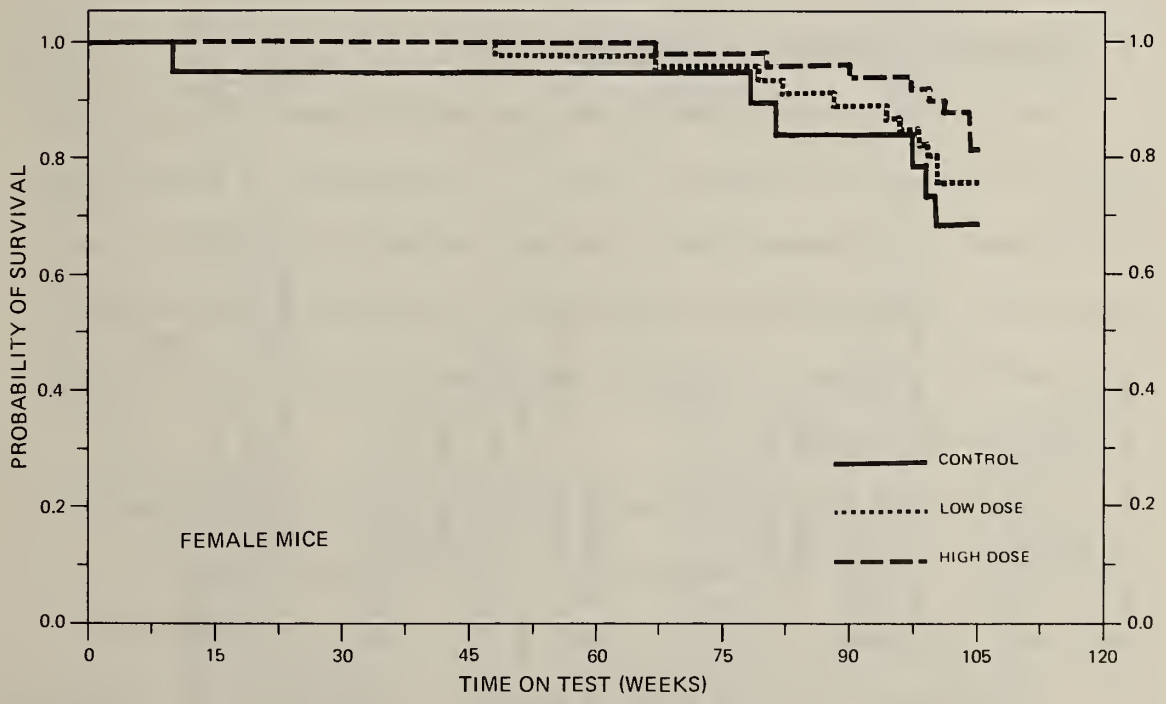


FIGURE 5
SURVIVAL COMPARISONS OF LITHOCHOLIC ACID CHRONIC STUDY MICE

A variety of tumors was observed in both the control and dosed groups. A few neoplasms occurred only, or with a greater frequency, in mice of dosed groups as compared with controls. The neoplasms which were observed have all been reported to occur spontaneously at similar incidences in this strain of mouse. No neoplasms were considered to be compound-related.

Nonneoplastic lesions were common in all groups. They were generally common chronic inflammatory, degenerative or fibrotic lesions, and none appeared to be compound-related.

Based on the results of this pathologic examination, lithocholic acid was not carcinogenic in male or female B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or lithocholic acid-dosed groups and where such tumors were observed in at least 5 percent of the group.

None of the statistical tests indicated a significant positive association between the administration of lithocholic acid and an increased incidence of tumors at any site for mice of either sex.

In male mice, the low dose to control Fisher exact test indicated a significant negative association between compound administration and

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH LITHOCHOLIC ACID[a]

TOPOGRAPHY:MORHPOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	1/20(0.05)	7/48(0.15)	5/46(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.917	2.174
Lower Limit	---	0.420	0.271
Upper Limit	---	128.374	100.415
Weeks to First Observed Tumor	105	97	104
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	7/20(0.35)	4/48(0.08)	7/47(0.15)
P Values[c]	N.S.	P = 0.011(N)	N.S.
Departure from Linear Trend[e]	P = 0.020	---	---
Relative Risk (Control)[d]	---	0.238	0.426
Lower Limit	---	0.060	0.154
Upper Limit	---	0.839	1.259
Weeks to First Observed Tumor	74	78	86
Liver: Hepatocellular Carcinoma[b]	5/20(0.25)	14/47(0.30)	6/47(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.191	0.511
Lower Limit	---	0.487	0.152
Upper Limit	---	3.765	1.916
Weeks to First Observed Tumor	86	53	76

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	6/20(0.30)	17/47(0.36)	9/47(0.19)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.206	0.638
Lower Limit	---	0.554	0.244
Upper Limit	---	3.263	1.939
Weeks to First Observed Tumor	86	53	76
Pancreatic Islets: Islet-Cell Adenoma[b]	2/19(0.11)	0/46(0.00)	0/44(0.00)
P Values[c]	P = 0.027(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.386	1.447
Weeks to First Observed Tumor	104	---	---

[a]Treated groups received doses of 125 or 250 mg/kg by gavage 3 times/week.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH LITHOCHOLIC ACID[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	0/19(0.00)	1/44(0.02)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.024	0.238
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	105
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	5/19(0.26)	17/45(0.38)	12/50(0.24)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.436	0.912
Lower Limit	---	0.619	0.360
Upper Limit	---	4.371	2.959
Weeks to First Observed Tumor	78	67	90
Liver: Hepatocellular Carcinoma[b]	0/18(0.00)	1/45(0.02)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.022	0.227
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	67

TABLE 6 (CONCLUDED)

[a]Treated groups received doses of 125 or 250 mg/kg by gavage 3 times/week.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

the incidence of a combination of leukemia and malignant lymphomas and the Cochran-Armitage test indicated a significant negative association between dosage and the incidence of islet-cell adenomas of the pancreas.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by lithocholic acid that could not be established under the conditions of this test.

V. DISCUSSION

There were no significant positive associations between the dosages of lithocholic acid administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Slight dose-related mean body weight depression was observed in male rats and female mice and high incidences of chronic kidney inflammation were observed in dosed female rats, indicating that the dosages of lithocholic acid administered to these animals in this bioassay may have approximated the maximum tolerated dosages. Since no mean body weight depression, relative to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of lithocholic acid to male mice, it is possible that these animals may have been able to tolerate a higher dosage. However, in the subchronic study there were deaths among all the dosed male mouse groups, even those receiving lithocholic acid at a level only twofold greater than the high dose utilized in the chronic bioassay.

None of the statistical tests for any site in rats or in mice of either sex indicated a significant positive association between compound administration and tumor incidence.

Under the conditions of this bioassay, lithocholic acid was not carcinogenic when administered by gavage to Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH LITHOCHOLIC ACID

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH LITHOCHOLIC ACID

	CONTROL (VEH) 11-1495	LOW DOSE 11-1493	HIGH DOSE 11-1491
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
PAPILLOMA, NOS	1 (5%)		
SEBACEOUS ADENOMA			1 (2%)
SEBACEOUS ADENOCARCINOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA	1 (5%)		1 (2%)
NEUROFIBROSARCOMA	1 (5%)		1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	4 (8%)	1 (2%)
PHEOCHROMOCYTOMA, METASTATIC			1 (2%)
NEUROFIBROSARCOMA, METASTATIC	1 (5%)		
HEMATOPOIETIC SYSTEM			
*#MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA,NOS		2 (4%)	1 (2%)
UNDIFFERENTIATED LEUKEMIA	2 (10%)	2 (4%)	1 (2%)
GRANULOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(20)	(48)	(49)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
#LYMPH NODE	(20)	(49)	(50)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
#LIVER	(19)	(49)	(50)
LEUKEMIA,NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (VEH) 11-1495	LOW DOSE 11-1493	HIGH DOSE 11-1491
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(49)	(50)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
HEPATOCELLULAR ADENOMA			2 (4%)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
#PANCREAS	(20)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
ACINAR-CELL ADENOMA		1 (2%)	1 (2%)
#STOMACH	(20)	(50)	(49)
PAPILLOMA, NOS			1 (2%)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
#SMALL INTESTINE	(20)	(50)	(49)
ADENOCARCINOMA, NOS			1 (2%)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
#COLON	(19)	(49)	(49)
ADENOCARCINOMA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
PHEOCHROMOCYTOMA, METASTATIC			1 (2%)
#URINARY BLADDER	(18)	(37)	(38)
PAPILLOMA, NOS	1 (6%)		
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(47)	(46)
CHROMOPHOBE ADENOMA		5 (11%)	6 (13%)
#ADRENAL	(20)	(50)	(50)
CORTICAL ADENOMA		2 (4%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONTINUED)

	CONTROL (VEH) 11-1495	LOW DOSE 11-1493	HIGH DOSE 11-1491
PHEOCHROMOCYTOMA	3 (15%)	7 (14%)	9 (18%)
PHEOCHROMOCYTOMA, MALIGNANT			3 (6%)
#THYROID	(9)	(39)	(27)
FOLLICULAR-CELL ADENOMA		2 (5%)	
FOLLICULAR-CELL CARCINOMA			1 (4%)
C-CELL ADENOMA		4 (10%)	1 (4%)
#PANCREATIC ISLETS	(20)	(50)	(50)
ISLET-CELL ADENOMA	2 (10%)	3 (6%)	2 (4%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(18)	(45)	(42)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
*SEMINAL VESICLE	(20)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
#TESTIS	(20)	(49)	(50)
INTERSTITIAL-CELL TUMOR	19 (95%)	45 (92%)	47 (94%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
NEUROFIBROSARCOMA		1 (2%)	
*MESENTERY	(20)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONCLUDED)

	CONTROL (VEH) 11-1495	LOW DOSE 11-1493	HIGH DOSE 11-1491
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	1 (2%)
DIAPHRAGM			
ADENOCARCINOMA, NOS, METASTATIC			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	3	6
MORIBUND SACRIFICE	2	2	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	45	40
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	47	48
TOTAL PRIMARY TUMORS	31	81	88
TOTAL ANIMALS WITH BENIGN TUMORS	19	45	48
TOTAL BENIGN TUMORS	28	73	72
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	7	12
TOTAL MALIGNANT TUMORS	3	7	14
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		3
TOTAL SECONDARY TUMORS	1		12
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	2
TOTAL UNCERTAIN TUMORS		1	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH LITHOCHOLIC ACID

	CONTROL (VEH) 11-1496	LOW DOSE 11-1494	HIGH DOSE 11-1492
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	44	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(50)
BASAL-CELL TUMOR			1 (2%)
*SUBCUT TISSUE	(20)	(49)	(50)
TRICHOEPITHELIOMA		1 (2%)	
FIBROMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
LEUKEMIA, NOS		3 (6%)	6 (12%)
UNDIFFERENTIATED LEUKEMIA	2 (10%)	5 (10%)	5 (10%)
GRANULOCYTIC LEUKEMIA		3 (6%)	2 (4%)
#MESENTERIC L. NODE	(20)	(46)	(47)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(49)
HEPATOCELLULAR ADENOMA		2 (4%)	2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (VEH) 11-1496	LOW DOSE 11-1494	HIGH DOSE 11-1492
#STOMACH	(20)	(48)	(50)
SARCOMA, NOS			1 (2%)
URINARY SYSTEM			
#URINARY BLADDER	(17)	(40)	(40)
TRANSITIONAL-CELL CARCINOMA			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(46)	(48)
CHROMOPHOBE ADENOMA	10 (50%)	18 (39%)	21 (44%)
#ADRENAL	(20)	(47)	(50)
PHEOCHROMOCYTOMA		3 (6%)	1 (2%)
#THYROID	(19)	(29)	(27)
C-CELL ADENOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(50)
FIBROADENOMA	1 (5%)	6 (12%)	6 (12%)
*MAMMARY LOBULE	(20)	(49)	(50)
PAPILLARY ADENOMA		1 (2%)	
#UTERUS	(20)	(48)	(50)
LEIOMYOSARCOMA	1 (5%)		
ENDOMETRIAL STROMAL POLYP	1 (5%)	7 (15%)	7 (14%)
#CERVIX UTERI	(20)	(48)	(50)
LEIOMYOMA		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(19)	(48)	(50)
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONTINUED)

	CONTROL (VEH) 11-1496	LOW DOSE 11-1494	HIGH DOSE 11-1492
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(50)
MESOTHELIOMA, METASTATIC		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	4	9
MORIBUND SACRIFICE	3	5	8
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	14	40	33
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONCLUDED)

	CONTROL (VEH) 11-1496	LOW DOSE 11-1494	HIGH DOSE 11-1492
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	34	35
TOTAL PRIMARY TUMORS	16	53	55
TOTAL ANIMALS WITH BENIGN TUMORS	11	30	26
TOTAL BENIGN TUMORS	12	42	39
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	11	16
TOTAL MALIGNANT TUMORS	4	11	16
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH LITHOCHOLIC ACID

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH LITHOCHOLIC ACID

	CONTROL (VEH) 22-2495	LOW DOSE 22-2493	HIGH DOSE 22-2491
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	3
ANIMALS NECROPSIED	20	48	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	48	47
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(48)	(47)
SEBACEOUS ADENOMA		1 (2%)	
FIBROSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(46)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	6 (13%)	5 (11%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(48)	(47)
MALIGNANT LYMPHOMA, NOS	1 (5%)	1 (2%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (10%)	2 (4%)	2 (4%)
LEUKEMIA,NOS	1 (5%)		
UNDIFFERENTIATED LEUKEMIA			1 (2%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
GRANULOCYTIC LEUKEMIA		1 (2%)	
MONOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(18)	(44)	(42)
HEMANGIOSARCOMA			1 (2%)
#MEDIASTINAL L.NODE	(20)	(46)	(42)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (2%)	
#MESENTERIC L. NODE	(20)	(46)	(42)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (10%)		1 (2%)
#SMALL INTESTINE	(19)	(46)	(46)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (VEH) 22-2495	LOW DOSE 22-2493	HIGH DOSE 22-2491
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(47)
HEPATOCELLULAR ADENOMA	1 (5%)	3 (6%)	3 (6%)
HEPATOCELLULAR CARCINOMA	5 (25%)	14 (30%)	6 (13%)
#BILE DUCT	(20)	(47)	(47)
BILE DUCT CARCINOMA	1 (5%)		
#STOMACH	(19)	(45)	(45)
SQUAMOUS CELL CARCINOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(12)	(34)	(32)
FOLLICULAR-CELL ADENOMA		1 (3%)	
#PANCREATIC ISLETS	(19)	(46)	(44)
ISLET-CELL ADENOMA	2 (11%)		
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONTINUED)

	CONTROL (VEH) 22-2495	LOW DOSE 22-2493	HIGH DOSE 22-2491
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(48)	(47)
HEMANGIOSARCOMA, METASTATIC			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	5	8
MORIBUND SACRIFICE		5	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	15	37	32
ANIMAL MISSING		2	3

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONCLUDED)

	CONTROL (VEH) 22-2495	LOW DOSE 22-2493	HIGH DOSE 22-2491
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	26	20
TOTAL PRIMARY TUMORS	17	31	23
TOTAL ANIMALS WITH BENIGN TUMORS	9	10	7
TOTAL BENIGN TUMORS	9	11	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	20	15
TOTAL MALIGNANT TUMORS	13	20	15
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH LITHOCHOLIC ACID

	CONTROL (VEH) 22-2496	LOW DOSE 22-2494	HIGH DOSE 22-2492
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	5	
ANIMALS NECROPSIED	19	45	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	19	45	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(19)	(45)	(50)
HEMANGIOSARCOMA		1 (2%)	
NEUROFIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(44)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	3 (6%)
FIBROSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(45)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		2 (4%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	3 (16%)	5 (11%)	2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (5%)	2 (4%)	
LEUKEMIA,NOS		1 (2%)	2 (4%)
UNDIFFERENTIATED LEUKEMIA			1 (2%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
GRANULOCYTIC LEUKEMIA		1 (2%)	1 (2%)
MONOCYTIC LEUKEMIA	1 (5%)	1 (2%)	
#SPLEEN	(16)	(41)	(49)
HEMANGIOSARCOMA		1 (2%)	
#LYMPH NODE	(18)	(43)	(47)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#MESENTERIC L. NODE	(18)	(43)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

	CONTROL (VEH) 22-2496	LOW DOSE 22-2494	HIGH DOSE 22-2492
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
#LIVER	(18)	(45)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(18)	(45)	(50)
HEPATOCELLULAR CARCINOMA		1 (2%)	3 (6%)
HEMANGIOSARCOMA			1 (2%)
#DUODENUM	(16)	(42)	(47)
ADENOMATOUS POLYP, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(14)	(42)	(43)
PHEOCHROMOCYTOMA			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(18)	(42)	(49)
HEMANGIOMA	1 (6%)		1 (2%)
#OVARY	(16)	(35)	(45)
GRANULOSA-CELL TUMOR		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONTINUED)

	CONTROL (VEH) 22-2496	LOW DOSE 22-2494	HIGH DOSE 22-2492
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(19)	(45)	(50)
LIPOMA			1 (2%)
ALL OTHER SYSTEMS			
THORAX			
FIBROSARCOMA			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	5	9
MORIBUND SACRIFICE	2	6	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	34	41
ANIMAL MISSING	1	5	

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONCLUDED)

	CONTROL (VEH) 22-2496	LOW DOSE 22-2494	HIGH DOSE 22-2492
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	20	23
TOTAL PRIMARY TUMORS	6	22	25
TOTAL ANIMALS WITH BENIGN TUMORS	1	1	7
TOTAL BENIGN TUMORS	1	1	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	18	17
TOTAL MALIGNANT TUMORS	5	20	18
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH LITHOCHOLIC ACID

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH LITHOCHOLIC ACID

	CONTROL (VEH) 11-1495	LOW DOSE 11-1493	HIGH DOSE 11-1491
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
INFLAMMATION, CHRONIC SUPPURATIV	1 (5%)		
RESPIRATORY SYSTEM			
#TRACHEA	(19)	(49)	(45)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, DIFFUSE			1 (2%)
ABSCESS, NOS			1 (2%)
#LUNG	(20)	(50)	(50)
CONGESTION, NOS		1 (2%)	
HEMORRHAGE			1 (2%)
PNEUMONIA, ASPIRATION	5 (25%)		
BRONCHOPNEUMONIA SUPPURATIVE			1 (2%)
BRONCHOPNEUMONIA, ACUTE		1 (2%)	
PNEUMONIA, CHRONIC MURINE	14 (70%)	18 (36%)	27 (54%)
METAPLASIA, OSSEOUS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(48)	(50)
HEMORRHAGE		1 (2%)	
#SPLEEN	(20)	(48)	(49)
AMYLOIDOSIS		1 (2%)	1 (2%)
HEMOSIDEROSIS		1 (2%)	
#MANDIBULAR L. NODE	(20)	(49)	(50)
LYMPHANGIECTASIS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (VEH) 11-1495	LOW DOSE 11-1493	HIGH DOSE 11-1491
*MESENTERIC L. NODE	(20)	(49)	(50)
LYMPHANGIECTASIS			2 (4%)
CIRCULATORY SYSTEM			
*HEART	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (5%)		1 (2%)
*HEART/ATRIUM	(20)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	1 (2%)
*MYOCARDIUM	(20)	(50)	(50)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, CHRONIC	5 (25%)	15 (30%)	10 (20%)
FIBROSIS	1 (5%)	7 (14%)	4 (8%)
DEGENERATION, NOS			2 (4%)
*CORONARY ARTERY	(20)	(50)	(50)
ARTERIOSCLEROSIS, NOS		1 (2%)	
*PANCREATIC ARTERY,	(20)	(50)	(50)
ARTERIOSCLEROSIS, NOS		1 (2%)	
*RENAL ARTERY	(20)	(50)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
NECROSIS, NOS		1 (2%)	
DIGESTIVE SYSTEM			
*LIVER	(19)	(49)	(50)
INFLAMMATION, CHRONIC			1 (2%)
NECROSIS, FOCAL			1 (2%)
AMYLOIDOSIS			1 (2%)
AMYLOIDOSIS, FOCAL			1 (2%)
HYPERPLASTIC NODULE			1 (2%)
HYPERPLASIA, FOCAL	3 (16%)	3 (6%)	1 (2%)
REGENERATIVE NODULE			1 (2%)
*LIVER/PERIPORTAL	(19)	(49)	(50)
FIBROSIS		3 (6%)	
*BILE DUCT	(19)	(49)	(50)
HYPERPLASIA, NOS	4 (21%)	12 (24%)	5 (10%)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (VEH) 11-1495	LOW DOSE 11-1493	HIGH DOSE 11-1491
#PANCREAS	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
ATROPHY, FOCAL		3 (6%)	
#PANCREATIC ACINUS	(20)	(50)	(50)
ATROPHY, NOS	1 (5%)	6 (12%)	
ATROPHY, FOCAL	2 (10%)		1 (2%)
#STOMACH	(20)	(50)	(49)
ULCER, NOS			1 (2%)
#SMALL INTESTINE	(20)	(50)	(49)
INFLAMMATION, NOS	1 (5%)		
#COLON	(19)	(49)	(49)
PARASITISM	10 (53%)	18 (37%)	27 (55%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
INFLAMMATION, CHRONIC	13 (65%)	36 (72%)	45 (90%)
INFLAMMATION, CHRONIC DIFFUSE			1 (2%)
#KIDNEY/TUBULE	(20)	(50)	(50)
CAST, NOS		1 (2%)	
#URINARY BLADDER	(18)	(37)	(38)
MINERALIZATION			1 (3%)
HEMORRHAGE	1 (6%)		
HEMORRHAGIC CYST			1 (3%)
INFLAMMATION, NOS			1 (3%)
ENDOCRINE SYSTEM			
#THYROID	(9)	(39)	(27)
HYPERPLASIA, C-CELL	1 (11%)		
#PANCREATIC ISLETS	(20)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(18)	(45)	(42)
INFLAMMATION, SUPPURATIVE		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONCLUDED)

	CONTROL (VEH) 11-1495	LOW DOSE 11-1493	HIGH DOSE 11-1491
*SEMINAL VESICLE	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (5%)		2 (4%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
ABSCESS, NOS	1 (5%)		
ATROPHY, PRESSURE			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
STEATITIS			1 (2%)
NECROSIS, FAT			1 (2%)
*PERITONEUM	(20)	(50)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
*PERICARDIUM	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVI		1 (2%)	
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC		3	
INFLAMMATION, GRANULOMATOUS		1	
NECROSIS, FAT	1	2	1
SPECIAL MORPHOLOGY SUMMARY			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH LITHOCHOLIC ACID

	CONTROL (VEH) 11-1496	LOW DOSE 11-1494	HIGH DOSE 11-1492
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(20)	(46)	(45)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)
#LUNG	(20)	(48)	(49)
PNEUMONIA, ASPIRATION	3 (15%)		
PNEUMONIA, CHRONIC MURINE	6 (30%)	8 (17%)	15 (31%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(48)	(49)
INFARCT, NOS			1 (2%)
HEMOSIDEROSIS			1 (2%)
HYPERPLASIA, LYMPHOID	1 (5%)		
#MANDIBULAR L. NODE	(20)	(46)	(47)
LYMPHANGIECTASIS	1 (5%)		
#MESENTERIC L. NODE	(20)	(46)	(47)
LYMPHANGIECTASIS		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(46)	(49)
INFLAMMATION, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

	CONTROL (VEH) 11-1496	LOW DOSE 11-1494	HIGH DOSE 11-1492
ABSCESS, NOS			1 (2%)
INFLAMMATION, CHRONIC		5 (11%)	5 (10%)
FIBROSIS	2 (10%)	1 (2%)	4 (8%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(43)	(44)
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	
METAPLASIA, SQUAMOUS		1 (2%)	
#LIVER	(20)	(49)	(49)
INFLAMMATION, NOS			2 (4%)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, NECROTIZING			1 (2%)
DEGENERATION, NOS			1 (2%)
NECROSIS, NOS			2 (4%)
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	1 (5%)	2 (4%)	5 (10%)
HYPERPLASIA, NODULAR		1 (2%)	
HYPERPLASIA, FOCAL		4 (8%)	7 (14%)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(49)	(49)
NECROSIS, NOS			1 (2%)
#LIVER/PERIPORTAL	(20)	(49)	(49)
FIBROSIS		1 (2%)	1 (2%)
FIBROSIS, FOCAL		1 (2%)	
#LIVER/HEPATOCYTES	(20)	(49)	(49)
HYPERPLASIA, FOCAL		3 (6%)	2 (4%)
#BILE DUCT	(20)	(49)	(49)
HYPERPLASIA, NOS	1 (5%)	8 (16%)	5 (10%)
#PANCREAS	(20)	(46)	(46)
ATROPHY, FOCAL		1 (2%)	
#PANCREATIC ACINUS	(20)	(46)	(46)
ATROPHY, NOS	1 (5%)	3 (7%)	2 (4%)
ATROPHY, FOCAL			1 (2%)
#STOMACH	(20)	(48)	(50)
ULCER, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (VEH) 11-1496	LOW DOSE 11-1494	HIGH DOSE 11-1492
#COLON	(20)	(47)	(48)
PARASITISM	9 (45%)	21 (45%)	17 (35%)
URINARY SYSTEM			
#KIDNEY	(20)	(48)	(50)
INFLAMMATION, CHRONIC	3 (15%)	23 (48%)	37 (74%)
NEPHROPATHY, TOXIC			1 (2%)
NEPHROSIS, CHOLEMIC			1 (2%)
INFARCT, ACUTE		1 (2%)	
PIGMENTATION, NOS			3 (6%)
#KIDNEY/CORTEX	(20)	(48)	(50)
CYST, NOS		1 (2%)	
#URINARY BLADDER	(17)	(40)	(40)
INFLAMMATION, NOS			1 (3%)
HYPERPLASIA, PAPILLARY			1 (3%)
#U.BLADDER/SUBMUCOSA	(17)	(40)	(40)
HEMORRHAGE		1 (3%)	
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(46)	(48)
CYST, NOS	2 (10%)	2 (4%)	3 (6%)
HEMORRHAGE	1 (5%)		
HYPERPLASIA, CHROMOPHOBE-CELL	1 (5%)		
#ADRENAL	(20)	(47)	(50)
INFARCT, NOS			1 (2%)
LIPOIDOSIS			1 (2%)
#ADRENAL MEDULLA	(20)	(47)	(50)
HYPERPLASIA, NOS	1 (5%)		
HYPERPLASIA, FOCAL	1 (5%)		
#THYROID	(19)	(29)	(27)
HYPERPLASIA, C-CELL		2 (7%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(50)
DILATATION/DUCTS		4 (8%)	3 (6%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (VEH) 11-1496	LOW DOSE 11-1494	HIGH DOSE 11-1492
INFLAMMATION, NOS		2 (4%)	1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)
HYPERPLASIA, NOS		1 (2%)	
*MAMMARY LOBULE	(20)	(49)	(50)
HYPERPLASIA, NOS		1 (2%)	
#UTERUS	(20)	(48)	(50)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE			3 (6%)
POLYPOID HYPERPLASIA			1 (2%)
#UTERUS/ENDOMETRIUM	(20)	(48)	(50)
CYST, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE			6 (12%)
INFLAMMATION, ACUTE			4 (8%)
ABSCESS, NOS		1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, CYSTIC		2 (4%)	3 (6%)
#OVARY	(20)	(47)	(50)
CYST, NOS	1 (5%)	3 (6%)	5 (10%)
INFLAMMATION, NOS			1 (2%)
ABSCESS, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(19)	(48)	(50)
INFLAMMATION, FOCAL		1 (2%)	
INFARCT, NOS			1 (2%)
ATROPHY, PRESSURE	3 (16%)	1 (2%)	5 (10%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(49)	(50)
INFLAMMATION, NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONCLUDED)

	CONTROL (VEH) 11-1496	LOW DOSE 11-1494	HIGH DOSE 11-1492
BODY CAVITIES			
*PLEURA	(20)	(49)	(50)
ABSCESS, NOS			1 (2%)
*MESENTERY	(20)	(49)	(50)
NECROSIS, FAT	1 (5%)		
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, NECROTIZING			1
INFLAMMATION, CHRONIC	1	1	1
INFLAMMATION, GRANULOMATOUS	1	1	
ROUND LIGAMENT			
INFLAMMATION, SUPPURATIVE			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH LITHOCHOLIC ACID

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH LITHOCHOLIC ACID

	CONTROL (VEH) 22-2495	LOW DOSE 22-2493	HIGH DOSE 22-2491
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	3
ANIMALS NECROPSIED	20	48	47
ANIMALS EXAMINED HISTOPATHCLOGICALLY **	20	48	47
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(46)
PNEUMONIA, CHRONIC MURINE	5 (25%)	2 (4%)	3 (7%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(44)	(42)
HEMATOPOIESIS			1 (2%)
#MESENTERIC L. NODE	(20)	(46)	(42)
CONGESTION, NOS	1 (5%)		
HEMORRHAGE	1 (5%)	1 (2%)	
HEMOSIDEROSIS	1 (5%)		
HYPERPLASIA, NOS			2 (5%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(48)	(47)
FIBROSIS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(47)
RUPTURE		1 (2%)	
HEMORRHAGE		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (VEH) 22-2495	LOW DOSE 22-2493	HIGH DOSE 22-2491
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
NECROSIS, FOCAL	1 (5%)		
AMYLOIDOSIS			1 (2%)
METAMORPHOSIS FATTY	1 (5%)	4 (9%)	1 (2%)
HEPATOCYTOMEGALY	1 (5%)		
ANGIECTASIS	1 (5%)		
#LIVER/PERIPORTAL	(20)	(47)	(47)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
#BILE DUCT	(20)	(47)	(47)
CYST, NOS			2 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		
#COLON	(16)	(46)	(45)
NEMATODIASIS		1 (2%)	
PARASITISM			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(48)	(47)
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)
#URINARY BLADDER	(18)	(45)	(42)
INFLAMMATION, CHRONIC			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(22)	(28)
HYPERPLASIA, NOS			1 (4%)
#ADRENAL	(12)	(38)	(38)
FIBROSIS, FOCAL			1 (3%)
#THYROID	(12)	(34)	(32)
COLLOID CYST	1 (8%)		
REPRODUCTIVE SYSTEM			
*PREPUCE	(20)	(48)	(47)
INFLAMMATION, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONCLUDED)

	CONTROL (VEH) 22-2495	LOW DOSE 22-2493	HIGH DOSE 22-2491
NERVOUS SYSTEM			
#BRAIN	(20)	(48)	(47)
CORPORA AMYLACEA		1 (2%)	1 (2%)
CALCIFICATION, FOCAL	2 (10%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(20)	(48)	(47)
INFLAMMATION, SUPPURATIVE			1 (2%)
*PERICARDIUM	(20)	(48)	(47)
INFLAMMATION, CHRONIC			1 (2%)
*MESENTERY	(20)	(48)	(47)
PERIARTERITIS		1 (2%)	
NECROSIS, FAT		1 (2%)	3 (6%)
ALL OTHER SYSTEMS			
CHEMIUM			
NECROSIS, FAT			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	15	16
ANIMAL MISSING/NO NECROPSY		2	3
AUTO/NECROPSY/HISTO PERF		4	1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH LITHOCHOLIC ACID

	CONTROL (VEH) 22-2496	LOW DOSE 22-2494	HIGH DOSE 22-2492
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	5	
ANIMALS NECROPSIED	19	45	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	45	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(19)	(44)	(50)
HEMORRHAGE	1 (5%)		
INFLAMMATION, INTERSTITIAL			1 (2%)
PNEUMONIA, ASPIRATION		1 (2%)	
PNEUMONIA, CHRONIC MURINE	2 (11%)	11 (25%)	9 (18%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(16)	(41)	(49)
HEMOSIDEROSIS		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIESIS	1 (6%)		1 (2%)
#MANDIBULAR L. NODE	(18)	(43)	(47)
HYPERPLASIA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(18)	(45)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (6%)		1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (VEH) 22-2496	LOW DOSE 22-2494	HIGH DOSE 22-2492
FIBROSIS, FOCAL		1 (2%)	
INFARCT, NOS			1 (2%)
HEMATOPOIESIS			2 (4%)
#LIVER/CENTRILOBULAR	(18)	(45)	(50)
NECROSIS, COAGULATIVE	1 (6%)		
#LIVER/KUPFFER CELL	(18)	(45)	(50)
HYPERPLASIA, NOS		1 (2%)	
#PANCREAS	(16)	(44)	(48)
CYSTIC DUCTS		1 (2%)	
FIBROSIS, DIFFUSE		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(18)	(45)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (6%)		
INFLAMMATION, CHRONIC		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(9)	(30)	(32)
CYSTIC FOLLICLES		1 (3%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(18)	(42)	(49)
PYOMETRA	4 (22%)	10 (24%)	7 (14%)
#UTERUS/ENDOMETRIUM	(18)	(42)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV			1 (2%)
#OVARY/OVIDUCT	(18)	(42)	(49)
CYST, NOS	1 (6%)		
#OVARY	(16)	(35)	(45)
CYST, NOS	3 (19%)	4 (11%)	5 (11%)
FOLLICULAR CYST, NOS			1 (2%)
HEMATOMA, NOS			1 (2%)
ATROPHY, NOS		1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONCLUDED)

	CONTROL (VEH) 22-2496	LOW DOSE 22-2494	HIGH DOSE 22-2492
NERVOUS SYSTEM			
#BRAIN	(18)	(44)	(50)
HYDROCEPHALUS, NOS	1 (6%)		
CALCIFICATION, FOCAL		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*VERTEBRA	(19)	(45)	(50)
FIBROUS OSTEODYSTROPHY	1 (5%)		
BODY CAVITIES			
*ABDOMINAL VISCERA	(19)	(45)	(50)
PERIARTERITIS			1 (2%)
*MESENTERY	(19)	(45)	(50)
STEATITIS		1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
PERIARTERITIS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	9	13
ANIMAL MISSING/NO NECROPSY	1	5	
AUTO/NECROPSY/HISTO PERF		1	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

Review of the Bioassay of Lithocholic Acid* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

August 31, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of Lithocholic Acid for carcinogenicity.

The primary reviewer agreed with the conclusion in the report that Lithocholic Acid was not carcinogenic, under the conditions of test. Although he indicated that there were too few control animals, it did not impact upon the interpretation of the study. He concluded that the bioassay was an adequate test for the carcinogenicity of Lithocholic Acid. He said that the data would indicate that Lithocholic Acid posed no unusual hazard with respect to human risk. He recommended that the report be accepted as written.

The secondary reviewer agreed that Lithocholic Acid was not carcinogenic, under the conditions of test. The major drawback to the study, he said, was some doubt that a maximum tolerated dose was tested. He stated that Lithocholic Acid would appear not to pose a direct risk as a human carcinogen. He recommended that the report on the bioassay of Lithocholic Acid be accepted.

A Subgroup member commented that the results were interesting since certain bile acids have been implicated in the etiology of colon cancer. A Program staff pathologist pointed out that a few intestinal tumors were observed among treated animals. Although they were not found in a

statistically significant incidence, he said that their spontaneous occurrence was relatively rare. One Clearinghouse member noted that Lithocholic Acid has been suggested to act as a tumor promoter.

A motion was approved unanimously that the report on the bioassay of Lithocholic Acid be accepted as written.

Members present were:

Arnold Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
(Verald Rowe, Dow Chemical, USA, submitted a written review)
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
(Kenneth Wilcox, Michigan State Health Department, submitted a written review)

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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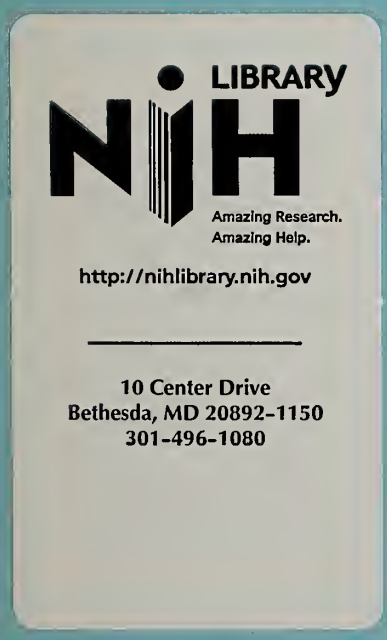

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